

## WHAT IS CLAIMED IS:

1. A protein preparation consisting essentially of an antigen that is immunologically reactive with a monoclonal antibody produced by the hybridoma cell line identified as ATCC No. HB9205, said preparation being substantially free of immunoglobulin G.
2. The protein preparation of claim 1, wherein said preparation has an amount of immunoglobulin G that is equal to or less than about 0.05% of the total protein of said preparation.
3. The protein preparation of claim 1, wherein said preparation has an amount of immunoglobulin G that is equal to or less than about 0.0015% of the total protein of said preparation.
4. The protein preparation of claim 1, wherein said preparation has less than about 500 pg of immunoglobulin G per  $\mu\text{g}$  of said antigen.
5. The protein preparation of claim 1, wherein said preparation has less than about 15 pg of immunoglobulin G per  $\mu\text{g}$  of said antigen.
6. A protein preparation consisting essentially of an antigen, which preparation is a diagnostic marker of Alzheimer's disease, wherein said antigen comprises a major polypeptide species that:
- (a) has an isoelectric point of about 6 in reduced or non-reduced form;
  - (b) binds to an affi-Blue column;
  - (c) is at least 50% soluble in a solution of 0.01 M sodium phosphate, 0.14 M sodium chloride and 1 mM phenyl methyl sulfonyl fluoride at pH 6.8, and precipitates in 50% saturated ammonium sulfate at 4°C;
  - (d) is immunologically reactive with a monoclonal antibody produced by the hybridoma cell line identified as ATCC No. HB9205; and
  - (e) is substantially free of immunoglobulin G.

7. The protein preparation of claim 6, wherein said preparation has an amount of immunoglobulin G that is equal to or less than about 0.05% of the total protein of said preparation.

8. The protein preparation of claim 6, wherein said preparation has an amount of immunoglobulin G that is equal to or less than about 0.0015% of the total protein of said preparation.

9. The protein preparation of claim 6, wherein said preparation has less than about 500 pg of immunoglobulin G per  $\mu\text{g}$  of said antigen.

10. The protein preparation of claim 6, wherein said preparation has less than about 15 pg of immunoglobulin G per  $\mu\text{g}$  of said antigen.

11. A process for obtaining the protein preparation of claim 1, said process comprising:

- (a) obtaining a sample of cortical brain tissue containing said antigen;
- (b) homogenizing said sample in buffer to obtain a homogenate;
- (c) removing particulate matter from said homogenate;
- (d) removing said antigen from said homogenate by contacting the homogenate with an antibody under conditions wherein said antigen and said antibody form an antigen-antibody complex;
- (e) eluting said antigen from said antigen-antibody complex; and
- (f) removing immunoglobulin G from the eluent to obtain said protein preparation.

12. The process of claim 11, wherein said immunoglobulin G is removed by incubation of said protein preparation with: (a) Protein A; (b) Protein G; (c) both Protein A and Protein G; or (d) an immunoglobulin G removal method that is substantially equivalent to (c).

13. In an improvement of a process for obtaining a preparation consisting essentially of an antigen that is immunologically reactive with the monoclonal antibody produced by the hybridoma cell line identified as ATCC No. HB9205, said improvement comprising removing immunoglobulin G from the antigen preparation  
5 to obtain a preparation that is substantially free of immunoglobulin G.

14. A method for detecting autoantibodies that are present in Alzheimer's disease comprising:

- 10 (a) obtaining a protein preparation according to claim 1, and a sample being tested for the presence of said autoantibodies;
- (b) electrophoresing said protein preparation on a gel;
- (c) transferring said electrophoresed protein preparation to a membrane;
- (d) contacting said membrane with a sample being tested for the presence of said autoantibodies such that an antigen-autoantibody complex can form; and
- 15 (e) detecting said autoantibodies by the formation of said complex.

15. The method of claim 14, wherein said sample is selected from the group consisting of cerebrospinal fluid, brain tissue homogenate/extract, urine, and blood.

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16. A method for detecting autoantibodies that are present in Alzheimer's disease comprising:

- (a) obtaining a protein preparation according to claim 1;
- (b) contacting said protein preparation with a sample being tested for the  
25 presence of said autoantibodies such that an antigen-autoantibody complex can form; and
- (c) detecting said autoantibodies by the formation of said complex.

17. The method of claim 16, wherein the presence of said autoantibodies is  
30 determined by the presence of said complex.

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18. The method of claim 16, wherein the amount of said complex is measured, and the amount of said autoantibodies is determined by the amount of said complex.

5 19. The method of claim 16, wherein said sample is selected from the group consisting of cerebrospinal fluid, brain tissue homogenate/extract, urine, and blood.

10 20. The method of claim 16, wherein said autoantibody is attached to a solid matrix.

15 21. The method of claim 16, further comprising the step of contacting said complex with an antibody that is immunologically reactive with an antigenic determinant found on either the autoantibody or the protein preparation such that an antigen-antibody or antibody-autoantibody complex is formed.

20 22. A method of increasing the ability of an Alzheimer's disease antigen to detect autoantibodies that are present in Alzheimer's disease, wherein said antigen is recombinant human tau, or tau isolated from various species including human, and said method comprises phosphorylating said antigens.

25 23. The method of claim 22, wherein said phosphorylation is done using a cell extract prepared from central nervous system cells that optionally has been treated with a phosphatase inhibitor such as okadaic acid.

24. The method of claim 22, wherein said phosphorylation is done using a purified or partially purified kinase selected from the group consisting of PKA, GSK, cdc2, cdc25, casein kinase I and II, MAP kinase, and PHF kinase.

30 25. A method of increasing the ability of an Alzheimer's disease antigen to detect autoantibodies that are present in Alzheimer's disease, wherein said antigen is

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tau isolated from various species including human, or is recombinant human tau, or is phosphorylated recombinant human tau or isolated tau, and said method comprises:

- (a) treating said antigen with hypericin, or calphostin C or the like; or
- (b) treating said antigen with free fatty acids; or
- 5 (c) treating said antigen with hydroxynonenal or other advanced glycation endproducts; or
- (d) combinations of the above.

26. The method of claim 25, wherein treatment is with free fatty acids, and  
10 said fatty acids are unsaturated fatty acids.

27. The method of claim 25, wherein treatment is with an advanced glycation endproduct, and said advanced glycation endproduct is the lipid peroxidation product 4-hydroxy-2-nonenal.

15 28. A monoclonal antibody that is immunologically reactive with an antibody directed against A68 antigen.

29. A method of obtaining an antibody that is immunologically reactive  
20 with an antibody directed against A68 antigen, said method comprising:

- (a) obtaining sera from individuals having high titers of anti-A68 autoantibodies, combining to create a pool, and isolating antibodies from said pool, or, obtaining isolated monoclonal antibodies to A68 antigen;
- (b) immunizing mice with said isolated antibodies;
- 25 (c) obtaining serum from said mice; and
- (d) testing said serum to identify mice having high levels of antibodies that are immunologically reactive with a monoclonal antibody or serum autoantibodies directed against A68 antigen.

30 30. The method of claim 29, which further comprises:

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(a) obtaining the spleens of said mice having high levels of antibodies that are immunologically reactive with a monoclonal antibody or serum autoantibodies directed against A68 antigen;

5 (b) fusing said spleens with myeloma cells and plating onto tissue culture plates;

(c) selecting for fused cells by HAT resistance; and

(d) testing said fused cells for production of antibodies that are immunologically reactive with a monoclonal antibody or serum autoantibodies directed against A68 antigen.

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31. The method of claim 30, which further comprises testing said fused cells for production of antibodies that are not immunologically reactive with a monoclonal antibody or serum autoantibodies which do not react with A68 antigen.

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32. A method for detecting autoantibodies that are present in Alzheimer's disease comprising:

(a) obtaining a protein preparation according to claim 1, a bovine microtubule associated protein preparation, and a sample being tested for the presence of said autoantibodies;

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(b) electrophoresing said protein preparation and said bovine microtubule associated protein preparation on separate lanes on a gel;

(c) transferring said electrophoresed protein preparation and said bovine microtubule associated protein preparation to a membrane;

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(d) contacting said membrane with a sample being tested for the presence of said autoantibodies such that an autoantibody complex can form with antigen present in said protein preparation and/or with antigen present in said bovine microtubule associated protein preparation; and

(e) detecting said autoantibodies by the formation of said complex(es).

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33. A method for detecting autoantibodies that are present in Alzheimer's disease comprising:

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(a) obtaining a protein preparation according to claim 1 or a bovine microtubule associated protein preparation;

(b) contacting said protein preparation or said bovine microtubule associated protein preparation with a sample being tested for the presence of said autoantibodies such that an antigen-autoantibody complex can form; and

(c) detecting said autoantibodies by the formation of said complex.

34. The method of claim 33, wherein the presence of said autoantibodies is determined by the presence of said complex.

35. The method of claim 33, wherein the amount of said complex is measured, and the amount of said autoantibodies is determined by the amount of said complex.

36. The method of claim 33, wherein said sample is selected from the group consisting of cerebrospinal fluid, brain tissue homogenate/extract, urine, and blood.

37. The method of claim 33, wherein said protein preparation or bovine microtubule associated protein preparation is attached to a solid matrix.

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